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The Council For Tobacco Research–U.S.A., Inc.

110 EAST 59TH STREET
NEW YORK, N.Y. 10022
(212) 421-8885

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JUL 29 1974

Application for Research Grant
(Use extra pages as needed)

Date: July 17, 1974

1. Principal Investigator (give title and degrees):
J. Alan Herd, M.D., Assoc. Professor of Psychobiology, Department of Psychiatry
Peter B. Dews, M.B., Ch.B., Ph.D., Stanley Cobb Professor of Psychiatry and Psychobiology
A. Clifford Barger, M.D., Robert Henry Pfeiffer Professor of Physiology

2. Institution & address:
New England Regional Primate Research Center
Laboratory of Psychobiology, Harvard Medical School
Department of Physiology, Harvard Medical School

3. Department(s) where research will be done or collaboration provided:
Department of Psychiatry
Department of Physiology

4. Short title of study:
Behavioral Hypertension and Arteriosclerosis in the Squirrel Monkey: Effects of Nicotine and Carbon Monoxide.

5. Proposed starting date: January 1, 1975.

6. Estimated time to complete: 3 years (January 1, 1975 to December 31, 1977).

7. Brief description of specific research aims:
(1) To determine the effects of blood pressure, diet, behavioral procedures, nicotine and carbon monoxide on entry, accumulation, and synthesis of cholesterol, triglycerides, and other solutes in carotid arteries, femoral arteries, coronary arteries, and the aorta in unanesthetized squirrel monkeys.

(2) To determine the effects of nicotine and carbon monoxide administered over long periods of time on blood pressure in the unanesthetized squirrel monkey during behavioral procedures.

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14. First year budget:

A. Salaries (give names or state "to be recruited")

	% time	Amount (incl. fringe benefits)
Professional (give % time of investigator(s) even if no salary requested)		
J. A. Herd		REDACTED
P. B. Dews		REDACTED
A. C. Barger		REDACTED
		REDACTED
Technical		
S. A. Grose		REDACTED

Sub-Total for A $ 27,000.00

B. Consumable supplies (by major categories)

Animal purchase; 100 rats @ $3.00	$ 300.00
18 Squirrel monkeys @ $150.00	2,700.00
Animal care, 12 monkeys @ $1.10 per diem	4,818.00
Radioisotopes	2,000.00
Physiological supplies	1,000.00
Electrical supplies	1,000.00
Surgical supplies	500.00
Pathology	1,000.00

Sub-Total for B $ 13,318.00

C. Other expenses (itemize)

Art work, photography, publication expenses	$1,500.00
Maintenance of equipment	1,000.00
Machine shop expenses	1,000.00
Travel (domestic)	500.00

Sub-Total for C $ 4,000.00

Running Total of A + B + C $ 44,318.00

D. Permanent equipment (itemize)

None

Sub-Total for D 0

E. Indirect costs (15% of A+B+C) E $ 6,648.00

Total request $50,966.00

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	$29,025.00	13,318.00	4,000.00	0	6,951.00	53,294.00
Year 3	31,202.00	13,318.00	4,000.00	0	7,278.00	55,798.00

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8. *Brief statement of working hypothesis:*

The level of systemic arterial blood pressure is the major determinant of the rates at which cholesterol and triglycerides accumulate in the media of large arteries in the squirrel monkey. Nicotine and carbon monoxide administered in small amounts over long periods of time have inconsequential effects on the long-term hypertensive and atherosclerotic responses to behavioral procedures and atherogenic diets.

9. Details of experimental design and procedures (append extra pages as necessary)

(1) Entry, accumulation, and synthesis of cholesterol, triglycerides, and other solutes in the walls of large arteries.

(a) experiments performed in vivo. Preliminary experiments will be conducted with rats and definitive experiments will be performed with squirrel monkeys.

(i) general procedure. Animals will be prepared with small plastic catheters implanted in the right internal iliac artery and vein. The rates at which various solutes accumulate in the walls of large arteries will be determined by infusing radioactively-labeled solutes intravenously and measuring the radioactivity in large arteries excised after several hours of infusion. Concentrations of radioactively-labeled solutes in arterial blood will be determined by measuring the radioactivity in blood samples through arterial catheters at various times throughout each experiments. Rates of infusion will be adjusted to maintain constant arterial concentrations of radioactively-labeled solutes for 2 to 4 hours. Radioactively-labeled solutes to be infused include ^{14}C- and ^{3}H-antipyrine, ^{14}C- and ^{3}H-sucrose, ^{14}C- and ^{3}H-inulin, ^{125}I-albumin, ^{14}C-cholesterol, and ^{3}H-acetate. Arteries to be excised and analyzed for radioactivity include carotid, femoral, and coronary arteries as well as segments of thoracic and abdominal aortas.

The rates at which radioactively-labeled solutes enter the walls of large arteries will be determined by infusing ^{14}C- and ^{3}H-labeled solutes in the same subject for different periods of time and by excising paired arteries one at a time at different times. For example, ^{3}H-inulin will be infused for 4 hours and ^{14}C-inulin will be infused from the 2nd to the 4th hours. The right carotid artery will be taken out after 3 hours of infusion and the left carotid artery will be taken out after 4 hours of infusion. In this way concentrations of ^{14}C- and ^{3}H-labeled inulin will be determined following 1, 2, 3, and 4 hours of infusion. The right carotid artery will be excised after 1 hour of infusion of ^{14}C-inulin and 3 hours of infusion of ^{3}H-inulin. The left carotid artery will be excised after 2 hours of infusion of ^{14}C-inulin and 4 hours of infusion of ^{3}H-inulin.

Localization of radioactivity in walls of arterial segments will be determined using autoradiography. ^{14}C-labeled solutes will be infused intravenously and paired arteries will be excised one at a time at different times, distended by intra lumenal pressure of 100 mm Hg, frozen, cut in cross section, and clamped against photographic film. By comparing autoradiographs prepared

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from identical arteries after different periods of infusion, it will be possible to determine the rates at which radioactively-labeled solutes move through each layer of the arterial wall.

Entry, accumulation, and synthesis of cholesterol and triglycerides in the walls of arteries will be determined using continuous intravenous infusions of ^{3}H-acetate and ^{14}C-cholesterol. After several hours of infusion, segments of arteries will be excised, homogenized, and extracted in chloroform-methanol solution. After evaporation of the chloroform and methanol the amounts of ^{14}C and ^{3}H extracted from each arterial segments will be measured as well as the total amount of lipid extracted. Samples of arterial blood will also be extracted in chloroform-methanol solution and amounts of ^{14}C and ^{3}H measured.

(ii) short-term experiments. In these studies control and experimental observations will be made during the same period of infusion. Each arterial segment will serve as its own control by infusing solutes labeled with ^{3}H and ^{14}C for different periods of time or comparisons will be made between results obtained using segments from right and left carotid or femoral arteries. Effects of high and low blood pressure on solute accumulation will be determined by cannulating one carotid or one femoral artery at its proximal end. Pressure in the cannulated artery will be lower than in the contralateral artery and blood will be allowed to flow in retrograde fashion through the artery out the proximal end through the cannula and into an adjacent vein. This procedure will serve to establish a continuous flow of arterial blood through the segment at reduced pressure. The effects of various agents on arterial permeability will be tested by infusing a solute labeled with ^{3}H during the control period, excising one of a pair of arteries, infusing the same solute labeled with ^{14}C while administering some agent such as carbon monoxide or nicotine and excising the contralateral artery after an appropriate period of time. Agents to be tested include norepinephrine and angiotensin II as well as carbon monoxide and nicotine.

(iii) long-term experiments. In these studies, control and experimental observations will be made on one of a pair of arteries excised at different times under different conditions. The rates at which various solutes accumulate in the walls of large arteries will be determined by infusing radioactively-labeled solutes intravenously and measuring the radioactivity in one artery excised under anesthesia after several hours of infusion without anesthesia. Following excision, the animal will be allowed to recover from surgery and anesthesia. Subsequently, the effects of behavioral hypertension, long-term administration of an atherogenic diet, chronic administration of carbon monoxide in the inspired air, and daily oral administration of nicotine will be determined. Radioactively-labeled solutes will again be administered by continuous intravenous infusion, arterial blood will be sampled intermittently, and the contralateral artery will be excised after the same period of infusion as was used during the control observation. A separate group of animals will be used to determine the effects of removing one carotid artery on entry and accumulation of solutes in the contralateral artery.

(b) experiments performed in vitro. The greatest difficulty encountered in the study of arterial segments perfused in vitro is to maintain the integrity of the endothelium. Arterial segments removed from the body frequently show swelling of endothelial cells and abnormalities of their nuclei within approximately 2 hours. In all our experiments, endothelial integrity will be assessed using electron micrographs. These experiments will be carried out in collaboration with Dr. Ramzi S. Cotran, Professor of Pathology at Harvard Medical School and The Peter Bent Brigham Hospital.

(i) general procedures. In vitro experiments will be performed with segments of carotid arteries, femoral arteries, and segments from the arch of the aorta which are free of small branches. Each segment will be tied at each end to soft plastic tubing and suspended in Krebs-Ringer bicarbonate buffer

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solution with glucose added to a concentration of 10 mM. The lumen will be filled with plasma that has been equilibrated with a mixture of oxygen in the ratio of 95/5 and the same mixture of gases will be bubbled continuously through the buffer solution surrounding the excised perfused artery. The tubing attached to each end of the arterial segment will be filled with plasma, connected to a strain gauge pressure transducer, and a microliter syringe. Static and dynamic pressures will be controlled by a pressure resevoir and a reciprocating syringe pump. At the beginning of each experiment, pressure-volume relations will be determined and a pressure-volume curve constructed. During each experiment , pressure and volume measurements will be repeated periodically and the fluid in the lumen of the artery will be replaced by fresh fluid from the syringe and tubing.

Bulk flow of water will be determined from the amount of fluid that must be added to the system to maintain pressure at initial values. Rates of flow will be determined at high and low pressures and during application of static and pulsatile pressures.

Rates of diffusion for solutes through the walls of arterial segments will be determined by perfusing the arterial lumen with fluid at atmospheric pressure containing known concentrations of radioactively-labeled solutes. The rates at which solutes diffuse through the arterial wall will be determined by measuring the amounts of radioactivity in fluid surrounding the arterial segment.

At the end of each experiment each arterial segment will be examined for endothelial integrity and tested for leaks. Before removing the artery from the perfusion system a suspension of ^{85}Sr-microspheres will be perfused at high pressure and amounts of radioactivity will be measured in the fluid surrounding the arterial segment. Endothelial integrity will be determined using electron micrographs.

(ii) short-term experiments. In these studies control observations and experimental interventions will be made during the same period of perfusion. Each arterial segment will serve as its own control or comparisons will be made between results obtained using right and left carotid or femoral arteries. During control periods bulk flow of water and movement of solutes through the arterial walls will be determined. Subsequently, the effects of high and low blood pressures, the effects of steady and pulsatile pressures, the effects of mechanical abrasion of the endothelium, the effects of hyaluronidase applied to the endothelium, and the effects of norepinephrine, angiotensin II, carbon monoxide, and nicotine in the perfusion fluid on arterial permeability to water and solutes will be determined.

(iii) long-term experiments. In these studies, control observations and experimental interventions will be made during different periods of perfusion with right and left carotid or femoral arteries. Animals will be anesthetized, one of a pair of arteries will be removed for perfusion under control conditions and then the animal will be allowed to recover from surgery and anesthesia. Subsequently, the effects of behavioral hypertension, long-term administration of an atherogenic diet, chronic administration of carbon monoxide in the inspired air, and daily administration of nicotine in the drinking water on arterial permeability to water and solutes will be determined. A separate group of animals will be used to determine the effects of removing one of a pair of arteries on permeability characteristics of the contralateral artery.

(2) Behavioral hypertension in the squirrel monkey during chronic administration of carbon monoxide and nicotine. Previous studies performed under a grant from the Council for Tobacco Research-U.S.A., Inc. (Grant No. CTR 843) demonstrated that nicotine administered subcutaneously to squirrel monkeys suppressed rates of responding on a lever under certain behavioral schedules. These studies will be continued and extended to include measurements of heart rate, blood pressure, and cardiac output during chronic administration of carbon monoxide

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in the inspired air and nicotine in the drinking water.

Behavioral schedules that exert persistent control over skeletal muscle movements also exert predictable and persistent effects on the cardiovascular system. Squirrel monkeys maintained for several months in daily sessions of schedules occasionally associated with delivery of noxious stimuli eventually develop sustained arterial hypertension. The effects on blood pressure of these daily sessions are not due simply to the delivery of noxious stimuli. All of the animals in training receive the greatest number of stimuli during the initial stages but at this time arterial pressures are not elevated above normal control values. As training progresses and each animal begins to press the lever more rapidly, the number of noxious stimuli delivered gradually decreases but mean arterial blood pressure rises. Continuous measurements of blood pressure 24 hours a day reveal that elevations of arterial blood pressure induced by behavioral techniques eventually persist between experimental sessions.

(i) general procedures. All animals to be studied will be prepared with systemic arterial and right atrial catheters for measurement of arterial blood pressure, right atrial pressure, and cardiac output. After recovery from surgery, each monkey will be conditioned to respond by pressing a lever under schedules in which lever pressing is reinforced by termination of a stimulus associated with the presentation of noxious stimuli. For example, under a fixed-ratio schedule, a fixed number of responses is required for reinforcement and under a fixed-interval schedule, the first response after a fixed time interval has elapsed is reinforced. If a monkey occasionally receives noxious stimuli in the presence of a light, and lever-pressing responses turn off the light, the animal will tend to respond by pressing the lever whenever the light is on. Thus, complex patterns of responding in the presence of a light can be maintained over long periods of time even though noxious stimuli are only infrequently delivered.

(ii) short-term experiments. Both conditional and unconditional cardiovascular responses to environmental stimuli will be studied. Animals will be acclimated to primate restraining chairs and isolated in temperature controlled chambers for a period of 16 hours before experiments begin. During this time, control measurements of heart rate, blood pressure, and cardiac output will be made. The onset of each experimental session will be signalled by turning on a small panel light located on the front of the restraining chair. Noxious electrical stimuli will be delivered occasionally in the presence of the panel light and will never be delivered in the absence of the light. The intensity of the noxious electrical stimuli and the frequency of their delivery will be adjusted systematically to determine the schedule of stimuli that has the maximum effect on heart rate and blood pressure. Some animals will be trained to press a lever to turn off the panel light and some will be untrained. As soon as stable performances and reproducible changes in cardiovascular function have been established, various ambient temperatures, various concentrations of oxygen, carbon dioxide, and carbon monoxide in inspired air, and administration of small amounts of nicotine infused intravenously will be tested for their effects on heart rate, blood pressure, cardiac output, and behavioral performances. Those schedules of environmental stimuli which have the greatest effects on cardiovascular function will be selected for further study of their long-term effect on arterial blood pressure and aortic atherosclerosis.

(iii) long-term experiments. Animals trained to press a lever to turn off a small panel light occasionally associated with delivery of noxious electrical stimuli eventually develop sustained elevations of arterial blood pressure that persist between daily experimental sessions. When sustained elevations in blood pressure have been induced using behavioral techniques, each animal with behavioral hypertension will be studied under various procedures to determine which schedules promote the return of blood pressure to control values and which

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cause behavioral hypertension to persist. Some animals will be left in their home cages for several weeks, some will be studied under extinction schedules, and some will be trained on schedule previously found to be associated with normal levels of blood pressure. In some of these hypertensive animals, the effects of carbon monoxide in the inspired air and nicotine in the drinking water administered continuously for several weeks will be studied for their effects on cardiovascular function and behavioral performances.

Eventually, some of the animals will develop elevated levels of arterial blood pressure that will not return to control values under any schedule of environmental stimuli. These animals with permanent hypertension will be fed an atherogenic diet with and without administration of carbon monoxide or nicotine. Effects of this regimen on aortic atherosclerosis in the squirrel monkey with behavioral hypertension will be determined by comparisons between the severity of atherosclerotic lesions seen in hypertensive animals and those seen in animals with normal blood pressure on the same regimen.

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10. Space and facilities available (when elsewhere than item 2 indicates, state location):

Several thousand square feet of office, laboratory, and animal care space are available in the New England Regional Primate Research Center and the Harvard Medical School. Fully equipped sterile surgical operating rooms, electrical and mechanical workshops and a photographic dark room are available for use on this project. Equipment available includes standard physiological recording equipment, behavioral programming and recording equipment, isolation chambers, primate restraining chairs, syringe infusion pumps, a cryostat microtome, a gamma scintillation spectrometer, a beta liquid scintillation spectrometer, and a PDP-8/L computer.

11. Additional facilities required:

None

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12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

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R: REDACTED MATERIAL

JAMES ALAN HERD

Born REDACTED

1956	M.D. University of British Columbia, Canada
1956-57	Intern, Vancouver General Hospital, Vancouver, British Columbia
1957-58	Assistant Resident in Medicine, Vancouver General Hospital
1958-59	Associate Resident in Pathology, Vancouver General Hospital
1959-60	Research Fellow in Medicine, Harvard Medical School Assistant in Medicine, Peter Bent Brigham Hospital Life Insurance Medical Research Fund Fellowship
1960-61	Research Fellow in Physiology, Harvard Medical School Life Insurance Medical Research Fund Fellowship
1961-62	Research Fellow in Physiology, Harvard Medical School Advanced Research Fellowship of the American Heart Assoc.
1962-63	Assistant Resident in Medicine, Peter Bent Brigham Hospital Webster-Underhill Fellowship
1963-64	Instructor in Physiology, Harvard Medical School
1963-68	Established Investigator of the American Heart Association
1964-67	Associate in Physiology, Harvard Medical School
1967-69	Assistant Professor of Physiology, Harvard Medical School
1969-73	Research Scientist Development Awardee, NIMH
1969-74	Associate Professor of Physiology, Harvard Medical School
1974-	Associate Professor of Psychobiology, Harvard Medical School

Memberships:

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R: REDACTED MATERIAL

PETER B. DEWS

Born REDACTED

1944	M.B. & Ch.B., University of Leeds, England
1951	Ph.D., University of Minnesota (in Physiology) Minneapolis
1959	M.A., Harvard University (hon.), Cambridge, Massachusetts
1944-45	House Physician, Grimsby & District General Hospital, England
1945-46	Demonstrator in Pharmacology, University of Leeds, England
1946-47	Lecturer in Pharmacology, University of Leeds, England
1948-49	Wellcome Research Fellow in Pharmacology, Wellcome Research Laboratories, Tuckahoe, New York
1950-51	Fellow and First Assistant in Physiology, Mayo Foundation, Section on Physiology
1952	Research Associate in Division of Biometry and Medical Statistics, Mayo Clinic
1953-54	Instructor in Pharmacology, Harvard Medical School
1954-56	Associate in Pharmacology, Harvard Medical School
1956-58	Assistant Professor of Pharmacology, Harvard Medical School
1959-62	Associate Professor of Pharmacology, Harvard Medical School
1962	Stanley Cobb Professor of Psychiatry & Psychobiology, Harvard Medical School

Memberships:

REDACTED

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R: REDACTED MATERIAL

A. CLIFFORD BARGER

Born [redacted]

U.S. Citizen

Born [redacted]

1939	A.B., Harvard University, Cambridge, Massachusetts
1943	M.D., Harvard University, Cambridge, Massachusetts
1938-41	Research Assistant (part-time), Harvard Fatigue Laboratory
1943	Medical House Officer, Peter Bent Brigham Hospital, Boston
1944-45	United States Army: Climatic Research Laboratory
1945	Assistant Resident in Medicine, Peter Bent Brigham Hospital
1946-47	Research Fellow in Physiology, Harvard Medical School
1946-53	Assistant in Medicine, Peter Bent Brigham Hospital, Boston
1948-50	Instructor in Physiology, Harvard Medical School
1950-53	Associate in Physiology, Harvard Medical School
1953	Associate Staff, Peter Bent Brigham Hospital, Boston
1953-55	Assistant Professor in Physiology, Harvard Medical School
1955-61	Associate Professor in Physiology, Harvard Medical School
1959	Consultant in Physiology (Medicine), Peter Bent Brigham Hospital, Boston
1959-60	Commonwealth Fund Fellow
1960	Consultant in Cardiovascular Physiology, World Health Organization
1961	Professor of Physiology, Harvard Medical School
1963	Robert Henry Pfeiffer Professor of Physiology, Harvard Medical School

Memberships:

[redacted]

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13. Publications (J. Alan Herd)

(1) Herd, J.A., Goodman, H.M. and Grose, S.A.: Blood flow rates through adipose tissues of unanesthetized rats. Am. J. Physiol. 214:263-268, 1968.

(2) Herd, J.A., Morse, W.H., Kelleher, R.T. and Jones, L.G.: Arterial hypertension in the squirrel monkey during behavioral experiments. Am. J. Physiol. 217:24-29, 1969.

(3) Benson, H., Herd, J.A., Morse, W.H. and Kelleher, R.T.: Behavioral induction of arterial hypertension and its reversal. Am. J. Physiol. 217:30-34, 1969.

(4) Herd, J.A.: The physiology of strong emotions: Cannon's scientific legacy re-examined. Physiologist 15:5-16, 1972.

(5) Jones, L.G. and Herd, J.A.: Autoradiographic visualization of ^{85}Kr in the normal dog kidney. Am. J. Physiol. 226:886-892, 1974.

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Specialized Center of Research in Hypertension	U.S.P.H.S. (HL 14150)	1,709,167	6/1/71-5/31/76
Central Control of Distribution of Organ Blood Flow	U.S.P.H.S. (HL 09154)	45,148	9/1/72-8/31/75
Behavioral Hypertension and Arteriosclerosis: Effects of Nicotine and Carbon Monoxide	Council for Tobacco Res. U.S.A. (CTR 843)	150,000	1/1/72-12/31/74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Central Control of Distribution of Organ Blood Flow	U.S.P.H.S. (HL 09154) Renewal Application	250,000 (Approx.)	9/1/75-8/31/80
Behavioral Influences on Cardiovascular Disease (Research Scientist Award)	U.S.P.H.S. (MH 13414)	142,500	1/1/75-12/31/79

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name J. Alan Herd, M.D.

Signature J. Alan Herd Date July 17, 1974

Telephone 617 (Area Code) 734-3300 (Number) 754 (Extension)

Checks payable to

Harvard Medical School, c/o Business Office

Mailing address for checks

25 Shattuck Street

Boston, Mass. 02115

Responsible officer of institution

Typed Name Henry C. Meadow

Title Executive Secretary, Committee on Research and Development

Signature Henry C. Meadow Date 7.19.74

Telephone 617 (Area Code) 734-3300 (Number) 441 (Extension)

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